The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,849,868 to Beer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

```
Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
1               5                  10                  15

Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
            20                  25                  30

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
        35                  40                  45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
    50                  55                  60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
65                  70                  75                  80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
                85                  90                  95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
            100                 105                 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
        115                 120                 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
    130                 135                 140

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
145                 150                 155                 160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
                165                 170                 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
            180                 185                 190

Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
        195                 200                 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
    210                 215                 220

Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225                 230                 235                 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
                245                 250                 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
            260                 265                 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
        275                 280                 285
```

```
Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
    290                 295                 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305                 310                 315                 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
                325                 330                 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
            340                 345                 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
        355                 360                 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
    370                 375                 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
385                 390                 395                 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
                405                 410                 415

Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
            420                 425                 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
        435                 440                 445
```

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

```
atgtcaattc ttacgcttaa caacaatacc tcgtcctcgc cgggtctgtt ccagtccggg   60
ggggacaacg ggcttggtgg tcataatgca aattctgcgt tggggcaaca acccatcgat  120
cggcaaacca ttgagcaaat ggctcaatta ttggcggaac tgttaaagtc actgctatcg  180
ccacaatcag gtaatgcggc aaccggagcc ggtggcaatg accagactac aggagttggt  240
aacgctggcg gcctgaacgg acgaaaaggc acagcaggaa ccactccgca gtctgacagt  300
cagaacatgc tgagtgagat gggcaacaac gggctggatc aggccatcac gcccgatggc  360
cagggcggcg ggcagatcgg cgataatcct ttactgaaag ccatgctgaa gcttattgca  420
cgcatgatgg acggccaaag cgatcagttt ggccaacctg gtacgggcaa caacagtgcc  480
tcttccggta cttcttcatc tggcggttcc ccttttaacg atctatcagg ggggaaggcc  540
ccttccggca actccccttc cggcaactac tctcccgtca gtaccttctc acccccatcc  600
acgccaacgt cccctacctc accgcttgat ttcccttctt ctcccaccaa agcagccggg  660
```

```
ggcagcacgc cggtaaccga tcatcctgac cctgttggta gcgcgggcat cggggccgga  720
aattcggtgg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc  780
gtgaaagcgg gtcaggtgtt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta  840
ggcgatggcg gccagtctga aaaccagaaa ccgctgttta tactggaaga cggtgccagc  900
ctgaaaaacg tcaccatggg cgacgacggg gcggatggta ttcatcttta cggtgatgcc  960
aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020
agcgcgggca aaaaatccca cgttgaaatc actaacagtt ccttcgagca cgcctctgac 1080
aagatcctgc agctgaatgc cgatactaac ctgagcgttg acaacgtgaa ggccaaagac 1140
tttggtactt ttgtacgcac taacggcggt caacagggta actgggatct gaatctgagc 1200
catatcagcg cagaagacgg taagttctcg ttcgttaaaa gcgatagcga ggggctaaac 1260
gtcaatacca gtgatatctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320
gccaacctga aggtggctga atga                                        1344
```

The above nucleotide and amino acid sequences are disclosed and further described in PCT Application Publication No. WO 99/07208 to Kim et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

```
Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1               5                  10                  15
Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
            20                  25                  30
Ser Lys Ala Leu Gln Glu Val Val Lys Leu Ala Glu Glu Leu Met
        35                  40                  45
Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
    50                  55                  60
Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65                  70                  75                  80
Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
                85                  90                  95
Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
            100                 105                 110
Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
        115                 120                 125
Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
    130                 135                 140
```

```
Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145                 150                 155                 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
                165                 170                 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
            180                 185                 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
        195                 200                 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
    210                 215                 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225                 230                 235                 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
                245                 250                 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
            260                 265                 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
        275                 280                 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
    290                 295                 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305                 310                 315                 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
                325                 330                 335

Asn Gln Ala Ala Ala
            340
```

This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 8 as follows:

```
atgcagagtc tcagtcttaa cagcagctcg ctgcaaaccc cggcaatggc ccttgtcctg    60

gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc   120

gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga   180

aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc   240
```

```
atcgctgcgc tggacaagct gatccatgaa aagctcggtg acaacttcgg cgcgtctgcg   300
gacagcgcct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc   360
aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac   420
gatatgccga tgctgaacaa gatcgcgcag ttcatggatg acaatcccgc acagtttccc   480
aagccggact cgggctcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac   540
gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag   600
agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcactccgag cagtttttcc   660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc   720
ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa   780
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgcagac cggtacgtcg   840
gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag   900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct   960
gcgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca  1020
gcctga                                                             1026
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,708,139 to Collmer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

```
Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
1               5                  10                  15
Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
            20                  25                  30
Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
        35                  40                  45
Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
    50                  55                  60
Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65                  70                  75                  80
Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
                85                  90                  95
Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
            100                 105                 110
Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
        115                 120                 125
```

```
Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
    130                 135                 140
Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145                 150                 155                 160
Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
                165                 170                 175
Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
            180                 185                 190
Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
        195                 200                 205
Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
    210                 215                 220
Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225                 230                 235                 240
Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
                245                 250                 255
Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
            260                 265                 270
Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
        275                 280                 285
Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
    290                 295                 300
Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305                 310                 315                 320
Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
                325                 330                 335
Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
            340                 345                 350
Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
        355                 360                 365
Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
    370                 375                 380
Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385                 390                 395                 400
Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
                405                 410                 415
Ala Ser Thr Gln His Thr Glu Leu
            420
```

This protein or polypeptide is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 10 as follows:

```
tccacttcgc tgattttgaa attggcagat tcatagaaac gttcaggtgt ggaaatcagg   60
ctgagtgcgc agatttcgtt gataagggtg tggtactggt cattgttggt catttcaagg  120
cctctgagtg cggtgcggag caataccagt cttcctgctg gcgtgtgcac actgagtcgc  180
aggcataggc atttcagttc cttgcgttgg ttgggcatat aaaaaaagga acttttaaaa  240
acagtgcaat gagatgccgg caaaacggga accggtcgct gcgctttgcc actcacttcg  300
agcaagctca accccaaaca tccacatccc tatcgaacgg acagcgatac ggccacttgc  360
tctggtaaac cctggagctg gcgtcggtcc aattgcccac ttagcgaggt aacgcagcat  420
gagcatcggc atcacacccc ggccgcaaca gaccaccacg ccactcgatt tttcggcgct  480
aagcggcaag agtcctcaac caaacacgtt cggcgagcag aacactcagc aagcgatcga  540
cccgagtgca ctgttgttcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga  600
cagcaccgtc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc  660
taaattgatc agtgcattga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa  720
gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcgggct  780
cggtacaccg tcggccgata gcgggggcgg cggtacaccg gatgcgacag gtggcggcgg  840
cggtgatacg ccaagcgcaa caggcggtgg cggcggtgat actccgaccg caacaggcgg  900
tggcggcagc ggtggcggcg gcacacccac tgcaacaggt ggcggcagcg gtggcacacc  960
cactgcaaca ggcggtggcg agggtggcgt aacaccgcaa atcactccgc agttggccaa 1020
ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc 1080
cggcaagatc aatgtggtga aagacaccat caaggtcggc gctggcgaag tctttgacgg 1140
ccacggcgca accttcactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca 1200
gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260
cgaggtcgat ggcatccacg tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320
gcatgcccag aacgtcggtg aagacctgat tacggtcaaa ggcgagggag gcgcagcggt 1380
cactaatctg aacatcaaga acagcagtgc caaaggtgca gacgacaagg ttgtccagct 1440
caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggt 1500
tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcatcgaagc 1560
taaccacggc aagttcgccc tggtgaaaag cgacagtgac gatctgaagc tggcaacggg 1620
caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgacccaaca 1680
caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc              1729
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 6,172,184 to Collmer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

```
Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1               5                  10                  15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
            20                  25                  30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
        35                  40                  45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
    50                  55                  60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65                  70                  75                  80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
                85                  90                  95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
            100                 105                 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
        115                 120                 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
    130                 135                 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145                 150                 155                 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
                165                 170                 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
            180                 185                 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
        195                 200                 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
    210                 215                 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225                 230                 235                 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
                245                 250                 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
            260                 265                 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
        275                 280                 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
    290                 295                 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305                 310                 315                 320
```

```
Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
                325                 330                 335

Gln Ser Thr Ser Thr Gln Pro Met
            340
```

Further information regarding this hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* is set forth in Arlat, M., et al., "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference in its entirety. It is encoded by a DNA molecule from *Pseudomonas solanacearum* having a nucleotide sequence corresponding SEQ. ID. No. 12 as follows:

```
atgtcagtcg gaaacatcca gagcccgtcg aacctcccgg gtctgcagaa cctgaacctc    60
aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgat caagcaggtc   120
gagaaggaca tcctcaacat catcgcagcc ctcgtgcaga aggccgcaca gtcggcgggc   180
ggcaacaccg gtaacaccgg caacgcgccg gcgaaggacg gcaatgccaa cgcgggcgcc   240
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc   300
ggcaacgtcg acgacgccaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa   360
gacctggtga agctgctgaa ggcggccctg cacatgcagc agcccggcgg caatgacaag   420
ggcaacggcg tgggcggtgc caacggcgcc aagggtgccg gcggccaggg cggcctggcc   480
gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcggcggcgg tgctggcgcc   540
ggcggcgcgg gtggcggtgt cggcggtgct ggtggcgcgg atggcggctc cggtgcgggt   600
ggcgcaggcg gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac   660
ggcccgcaga acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac   720
cagggcggcc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctggtgcag   780
atgatgcagc aaggcggcct cggcggcggc aaccaggcgc agggcggctc gaagggtgcc   840
ggcaacgcct cgccggcttc cggcgcgaac ccgggcgcga accagcccgg ttcggcggat   900
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc   960
gtccagatcc tgcagcagat gctggcggcg cagaacggcg gcagccagca gtccacctcg  1020
acgcagccga tgtaa                                                   1035
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,776,889 to Wei et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor polypeptide or protein derived from X*anthomonas campestris* has an amino acid sequence corresponding to SEQ. ID. No. 13 as follows:

```
Met Asp Ser Ile Gly Asn Asn Phe Ser Asn Ile Gly Asn Leu Gln Thr
 1               5                  10                  15

Met Gly Ile Gly Pro Gln Gln His Glu Asp Ser Ser Gln Gln Ser Pro
            20                  25                  30

Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln Leu Leu Ala Met Phe Ile
        35                  40                  45

Met Met Met Leu Gln Gln Ser Gln Gly Ser Asp Ala Asn Gln Glu Cys
    50                  55                  60

Gly Asn Glu Gln Pro Gln Asn Gly Gln Gln Glu Gly Leu Ser Pro Leu
65                  70                  75                  80

Thr Gln Met Leu Met Gln Ile Val Met Gln Leu Met Gln Asn Gln Gly
                85                  90                  95

Gly Ala Gly Met Gly Gly Gly Gly Ser Val Asn Ser Ser Leu Gly Gly
            100                 105                 110

Asn Ala
```

This hypersensitive response elicitor polypeptide or protein has an estimated molecular weight of about 12 kDa based on the deduced amino acid sequence, which is consistent with a molecular weight of about 14 kDa as detected by SDS-PAGE. The above protein or polypeptide is encoded by a DNA molecule according to SEQ. ID. No. 14 as follows:

```
atggactcta tcggaaacaa cttttcgaat atcggcaacc tgcagacgat gggcatcggg  60
cctcagcaac acgaggactc cagccagcag tcgccttcgg ctggctccga gcagcagctg 120
gatcagttgc tcgccatgtt catcatgatg atgctgcaac agagccaggg cagcgatgca 180
aatcaggagt gtggcaacga acaaccgcag aacggtcaac aggaaggcct gagtccgttg 240
acgcagatgc tgatgcagat cgtgatgcag ctgatgcaga accagggcgg cgccggcatg 300
ggcggtggcg gttcggtcaa cagcagcctg ggcggcaacg cc                    342
```

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent Application Serial No. 09/829,124, which is hereby incorporated by reference in its entirety.

Other embodiments of the present invention include, but are not limited to, use of a hypersensitive response elicitor protein or polypeptide derived

from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference in its entirety. A hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference in their entirety.

The hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet, et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor for use in accordance with the present invention is derived from *Clavibacter michiganensis* subsp. *sepedonicus*. The use of this particular hypersensitive response elicitor is described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference in its entirety.

Other elicitors can be readily identified by isolating putative hypersensitive response elicitors and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art.

The hypersensitive response elicitor protein or polypeptide can also be a fragment of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding editions), which are hereby incorporated by reference in their entirety. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference in its entirety.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference in its entirety. These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora*

hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. DNA molecules encoding these fragments can also be utilized in the chimeric gene of the present invention.

DNA molecules encoding a hypersensitive response elicitor protein or polypeptide can also include a DNA molecule that hybridizes under stringent conditions to the DNA molecule having nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, 8, 10, 12, or 14. An example of suitable stringency conditions is when hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the temperature for either hybridization or washing conditions or increasing the sodium concentration of the hybridization or wash medium. Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 μg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

Variants of suitable hypersensitive response elicitor proteins or polypeptides can also be expressed. Variants may be made by, for example, the deletion, addition, or alteration of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

When it is desirable to perform the methods of the present invention with application of the hypersensitive response elicitor protein or polypeptide to a plant seed or a plant, it is preferable, though not necessary, that the hypersensitive response elicitor protein or polypeptide be applied in isolated form or with a carrier as discussed hereinafter.

One particular hypersensitive response elicitor protein, known as $harpin_{Ea}$, is commercially available from Eden Bioscience Corporation (Bothell, Washington) under the name of Messenger®. Messenger® contains 3% by weight of $harpin_{Ea}$ as the active ingredient and 97% by weight inert ingredients. $Harpin_{Ea}$ is one type of hypersensitive response elicitor protein from *Erwinia amylovora*, identified herein by SEQ. ID. No. 3.

Alternatively, the hypersensitive response elicitor protein or polypeptide can be recombinantly produced, isolated, and then purified, if necessary. When recombinantly produced, the hypersensitive response elicitor protein or polypeptide is expressed in a recombinant host cell, typically, although not exclusively, a prokaryote.

When a prokaryotic host cell is selected for subsequent transformation, the promoter region used to construct the recombinant DNA molecule (i.e., transgene) should be appropriate for the particular host. The DNA sequences of eukaryotic promoters, as described *infra* for plants, differ from those of prokaryotic promoters. Eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a prokaryotic system, and, further, prokaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of mRNA in prokaryotes depends upon the presence of the proper prokaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in prokaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference in its entirety.

Promoters vary in their "strength" (i.e., their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *rec*A promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operons, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific

messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires a Shine-Dalgarno ("SD") sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include, but are not limited to, the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the DNA molecule coding for a hypersensitive response elicitor protein or polypeptide has been ligated to its appropriate regulatory regions using well known molecular cloning techniques, it can then be introduced into a vector or otherwise introduced directly into a host cell (Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference in its entirety).

The recombinant molecule can be introduced into host cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like. Preferably the host cells are either a bacterial cell or a plant cell. The host cells, when grown in an appropriate medium, are capable of expressing the hypersensitive response elicitor protein or polypeptide, which can then be isolated therefrom and, if necessary, purified.

The hypersensitive response elicitor protein or polypeptide of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is produced but not secreted into the growth medium of recombinant host cells, usually although not exclusively bacterial host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium.

In the case of an unsecreted hypersensitive response elicitor protein or polypeptide, the protein or polypeptide can be isolated from the host cell (e.g., *E. coli*)

carrying a recombinant plasmid by lysing the host cell with sonication, heat, or chemical treatment, after which the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the hypersensitive response elicitor protein is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

Alternatively, it is desirable for recombinant host cells to secrete the hypersensitive response elicitor protein or polypeptide into growth medium, thereby avoiding the need to lyse cells and remove cellular debris. To enable the host cell to secrete the hypersensitive response elicitor, the host cell can also be transformed with a type III secretion system in accordance with Ham et al., "A Cloned *Erwinia chrysanthemi* Hrp (Type III Protein Secretion) System Functions in *Escherichia coli* to Deliver *Pseudomonas syringae* Avr Signals to Plant Cells and Secrete Avr Proteins in Culture," Microbiol. 95:10206-10211 (1998), which is hereby incorporated by reference in its entirety. After growing recombinant host cells which secrete the hypersensitive response elicitor into growth medium, isolation of the hypersensitive response elicitor protein or polypeptide from growth medium can be carried out substantially as described above.

The methods of the present invention which involve application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, dusting, and leaf abrasion proximate to when elicitor application takes place. More than one application of the hypersensitive response elicitor protein or polypeptide may be desirable either to realize maximal benefit of the value-added trait or overcome a yield penalty, particularly over the course of a growing season. When treating plant seeds in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, dusting, or injection. Other suitable application procedures can be

envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may also be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide. Such propagated plants may, in turn, be useful in producing seeds or propagules (e.g., cuttings) that produce plants capable of insect control.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, herbicide, and mixtures thereof. Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

Although application of the hypersensitive response elicitor protein or polypeptide is preferably carried out in isolated form or with a carrier, the hypersensitive response elicitor protein or polypeptide can also be applied in a non-isolated but non-infectious form. When applied in non-isolated but non-infectious

form, the hypersensitive response elicitor is applied indirectly to the plant via application of a bacteria which expresses and then secretes or injects the expressed hypersensitive response elicitor protein or polypeptide into plant cells or tissues. Such application can be carried out by applying the bacteria to all or part of a plant or a plant seed under conditions where the polypeptide or protein contacts all or part of the cells of the plant or plant seed. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to enhance plant growth, impart stress tolerance in plants, impart disease resistance in plants, and/or to effect insect control.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed in a non-isolated but non-infectious form can be carried out in a number of ways, including: 1) application of bacteria which do not cause disease and are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein, and 2) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example, *E. coli*, which does not elicit a hypersensitive response in plants, can be transformed with genes encoding a hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species other than *E. coli* can also be used in this embodiment of the present invention.

In another embodiment of the bacterial application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment, these bacteria are applied to plants or their seeds which are not susceptible to the disease carried by the bacteria. For example, *Erwinia amylovora* causes disease in apple or pear but not in tomato. However, such bacteria will elicit a hypersensitive response in tomato. Accordingly, in accordance with this embodiment of the present invention, *Erwinia amylovora* can

be applied to tomato plants or seeds to enhance growth without causing disease in that species.

Another aspect of the present invention is a method which is carried out by providing a plant cell, transforming the plant cell with (i) a first DNA molecule encoding a transcript or a protein or polypeptide which confers a trait to a plant grown from the transformed plant cell and (ii) a second DNA molecule encoding a hypersensitive response elicitor protein or polypeptide which is different than the protein or polypeptide encoded by the first DNA molecule, the transforming being carried out under conditions effective to produce a transformed plant cell, and then regenerating a transgenic plant from the transformed plant cell. By transforming the plant cell with the second DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, as discussed *infra*, the resulting transgenic plant expresses the hypersensitive response elicitor and exhibits enhanced growth, stress tolerance, disease resistance, or insect resistance.

According to one embodiment, transforming with the second DNA molecule imparts enhanced growth, stress tolerance, disease resistance, or insect resistance to the plant, thereby maximizing benefit to the plant of the trait conferred by transforming with the first DNA molecule. For example, when the particular trait conferred by the first DNA molecule relates to specific but limited growth enhancement, stress tolerance, disease resistance, or insect resistance of a transgenic plant, this embodiment relates to conferring broad growth enhancement, stress tolerance, disease resistance, or insect resistance that complements the specific but limited trait.

According to another embodiment, transforming with the first DNA molecule is accompanied by a deleterious effect on growth, stress tolerance, disease resistance, or insect resistance, and transforming with the second DNA molecule overcomes the deleterious effect. Thus, this aspect of the present invention is also directed to overcoming a yield penalty resulting from a trait.

Any of the above-described DNA molecules encoding a hypersensitive response elicitor protein or polypeptide can be used to prepare a desired transgenic plant that expresses both a transgene conferring a value-added trait and a transgene encoding a hypersensitive response elicitor.

The transgene or DNA molecule conferring a trait can be any DNA molecule that confers a value-added trait to a transgenic plant. The value-added trait can be for disease resistance, insect resistance, enhanced growth, herbicide resistance, stress tolerance, male sterility, modified flower color, or biochemically modified plant product. Biochemically modified plant products can include, without limitation, modified cellulose in cotton, modified ripening of fruits or vegetables, modified flavor of fruits or vegetables, modified flower color, expression of industrial enzymes, modified starch content, modified dietary fiber content, modified sugar metabolism, modified food quality or nutrient content, and bioremediation.

The transgene or DNA molecule conferring a value-added trait can encode either a transcript (sense or antisense) or a protein or polypeptide which is different from the hypersensitive response elicitor protein or polypeptide. Either the transcript or the protein or polypeptide, or both, can confer the value-added trait.

A number of proteins or polypeptides which can confer a value-added trait are known in the art and others are continually being identified, isolated, and expressed in host plants. Suitable proteins or polypeptides which can be encoded by the transgene or DNA molecule conferring a value-added trait include, without limitation, B.t. toxin, *Photorhabdus luminescens* protein, protease inhibitors, amylase inhibitors, lectins, chitinases, endochitinase, chitobiase, defensins, osmotins, crystal proteins, virus proteins, herbicide resistance proteins, mannitol dehydrogenase, PG inhibitors, ACC degradation proteins, barnase, phytase, fructans, invertase, and SAMase.

A number of transcripts which can confer a value-added trait are known in the art and others are continually being identified, isolated, and expressed in host plants. The transcript encoded by the transgene or DNA molecule conferring a trait can be either a sense RNA molecule, which is translatable or untranslatable, or an antisense RNA molecule capable of hybridizing to a target RNA or protein. Suitable transcripts which can be encoded by the transgene or DNA molecule conferring a trait include, without limitation, translatable and untranslatable RNA transcripts capable of interfering with plant virus pathogenesis (de Haan et al., "Characterization of RNA-Mediated Resistance to Tomato Spotted Wilt Virus in Transgenic Tobacco Plants," BioTechnology 10:1133-1137 (1992); Pang et al., "Nontarget DNA

Sequences Reduce the Transgene Length Necessary for RNA-Mediated Tospovirus Resistance in Transgenic Plants," Proc. Natl. Acad. Sci. USA94:8261-8266 (1997), which are hereby incorporated by reference in their entirety) and antisense RNA molecules which interfere with the activity of an enzyme (e.g., starch synthase, ACC oxidase, pectinmethylesterase, polygalacturonase, etc.) or the synthesis of a particular product (e.g., glycoalkaloid synthesis).

Exemplary expression products of the transgene or DNA molecule conferring a trait and their uses are identified in Table 1 below.

Table 1: Expression Products of Transgene Conferring Value-Added Trait and Their Uses

Trait and Expression Product	Reference
Pest/Pathogen Resistance	
B.t. toxin	U.S. Patent No. 5,990,383 to Warren et al.
crystal proteins	U.S. Patent No. 4,996,155 to Sick et al.
Photorhabdus luminescens protein	Bowen et al., Science 280:2129 (1998)
protease inhibitors	Ryan, Annu. Rev. Phytopathol. 38:425-449 (1990)
amylase inhibitors	Mundy et al., Planta 169:51-63 (1986)
lectins	EP Patent Application No. 351,924 A to Shell
chitinase (nematode & fungal)	U.S. Patent No. 5,290,687 to Suslow et al.
endochitinase & chitobiase	U.S. Patent No. 5,378,821 to Harman et al.
endochitnase activity	U.S. Patent No. 5,446,138 to Blaiseu et al
defensins	U.S. Patent No. 4,705,777 to Lehrer et al.
osmotins	Liu et al., PNAS USA 91:1888 (1994)
tobacco mosaic virus coat protein	Beachy et al., Rev. Phytopathol. 28:451-474 (1990)
cucumber mosaic virus coat protein	U.S. Patent No. 5,349,128 to Quemada et al.
potato coat protein	U.S. Patent No. 4,970,168 to Tumer et al.
potato leaf roll virus coat protein	U.S. Patent No. 5,304,730 to Lawson et al.
potato virus replicase	U.S. Patent No. 5,503,999 to Jilka et al. U.S. Patent No. 5,510,253 to Mitsky et al.
potyvirus coat protein	WO 90/02184 to Gonsalves et al.
Herbicide Resistance	
glyphosate resistance (EPSP synthase protein)	U.S. Patent No. 4,535,060 to Comai et al.
chlorsulfuron resistance	Haughn et al., Mol. Gen. Genet. 211:266 (1988)
phosphinothriun/bialaphos resistance	De Block, EMBO J. 6:2513 (1987)
Improved Nutrient Content	
protein	U.S. Patent No. 6,057,493 to Willmitzer et al.
vitamins	U.S. Patent No. 5,750,872 to Bennett et al.
oils	Shintani et al., Plant Physiol. 114(3):881-886 (1997); U.S. Patent No. 6,069,298 to Gengenbach et al.

Table 1 cont.

Trait and Expression Product	Reference
Stress Tolerance	
cold	U.S. Patent No. 5,891,859 to Thomashow et al.
metals	U.S. Patent No. 5,668,294 to Meaghar et al.
drought	U.S. Patent No. 5,563,324 to Tarczynski et al. U.S. Patent No. 5,780,709 to Adams et al.
Secondary Compounds	
PHB	Poirier et al., Science 256:520 (1992); Poirier et al., Bio/Technology 13:142 (1995)
antibodies	Tavladorki et al., Nature 366:469 (1993)
pharmaceutical peptides	EP Patent Application No. 436,003 A to Sijmons et al.
Improved Fiber	
cotton	U.S. Patent No. 5,932,713 to Kasukabe et al.
Modified Ripening	
PG inhibition	U.S. Patent No. 5,942,657 to Bird et al.
block ethylene synthesis: ACC	U.S. Patent No. 5,723,766 to Theologis et al.;
degradation	U.S. Patent No. 5,886,164 to Bird et al.
S-adenosylmethionine hydrolase	U.S. Patent No. 5,723,746 to Bestwick et al.
Male Sterility	
barnase (*Bacillus amyloliquefaciens*)	Hartley, J. Mol. Biol. 202:913 (1988)
ribonucleases (RNase T1 from *Asperqillus oryzae*)	EP Patent No. 344,029 to Mariani et al.
Industrial Enzymes	
phytase	U.S. Patent No. 5,593,963 to Van Ooijen et al.; Van Hartingsveldt et al., Gene 127:87 (1993)
Flower Color	
pH gene products	U.S. Patent No. 5,534,660 to Chuck et al. U.S. Patent No. 5,910,627 to Chuck et al.
dihydroflavonol 4-reductase	U.S. Patent No. 5,410,096 to Meyer et al.
flavonoid biosynthetic pathway gene	U.S. Patent No. 5,034,323 to Jorgensen et al.
Starch Content	
anti-sense starch synthase	U.S. Patent No. 6,057,493 to Willmitzer et al.
amylose content	U.S. Patent No. 6,066,782 to Kossman et al.
Dietary Fiber	
potato increased fructans	U.S. Patent No. 5,986,173 to Smeekens et al.
Improved Flavor	
alcohol dehydrogenase II	U.S. Patent No. 6,011,199 to Speirs et al.
pH gene products	U.S. Patent No. 5,534,660 to Chuck et al. U.S. Patent No. 5,910,627 to Chuck et al.
sweetness (monellin/thaumatin)	U.S. Patent No. 5,739,409 to Fischer et al.

Table 1 cont.

Trait and Expression Product	Reference
Bioremediation	
metalothionein in *Brassicaceae*	U.S. Patent No. 5,364,451 to Raskin et al.
Modified Sugar Metabolism	
invertase	U.S. Patent No. 5,917,127 to Willmitzer et al.
Modified Food Quality	
altered carbohydrate composition	WO 90/12876 to Gausing et al.
increased glutenin (wheat & others)	U.S. Patent No. 5,914,450 to Blechl et al.
increased storage lipids in seed	U.S. Patent No. 5,914,449 to Murase et al.

Each of the references listed in Table 1 is hereby incorporated by reference in its entirety.

To express, in plant tissues, the DNA molecule encoding a hypersensitive response elicitor protein or polypeptide and/or the DNA molecule conferring a value-added trait, the coding regions must be ligated to appropriate regulatory regions which are operable in plant tissues. Therefore, plant expressible promoters and 3' polyadenylation regions must be ligated to the DNA molecules to afford a transgene which can then be used to transform plant cells or tissues.

Any plant-expressible promoter can be utilized regardless of its origin, i.e., viral, bacterial, plant, etc. Without limitation, two suitable promoters include the nopaline synthase promoter (Fraley et al., "Expression of Bacterial Genes in Plant Cells," Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 35S promoter (O'Dell et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety). Both of these promoters yield constitutive expression of coding sequences under their regulatory control.

While constitutive expression is generally suitable for expression of transgenes, it should be apparent to those of skill in the art that temporally or tissue regulated expression may also be desirable, in which case any regulated promoter can be selected to achieve the desired expression. Typically, the temporally or tissue regulated promoters will be used in connection with DNA molecules that are expressed at only certain stages of development or only in certain tissues.

For example, the E4 and E8 promoters of tomato have been used to direct fruit-specific expression of a DNA sequence in transgenic tomato plants

(Cordes et al., Plant Cell 1:1025-1034 (1989); Deikman et al., EMBO J. 7:3315-3320 (1988); and Della Penna et al., Proc. Natl. Acad. Sci. USA 83:6420-6424 (1986), which are hereby incorporated by reference in their entirety). Another fruit-specific promoter is the PG promoter (Bird et al., Plant Molec. Biol. 11:651-662 (1988), which is hereby incorporated by reference). Another tissue-specific promoter is the AP2 promoter from the ovule-specific BEL1 gene promoter described in Reiser et al., Cell 83:735-742 (1995), which is hereby incorporated by reference in its entirety.

Promoters useful for expression in seed tissues include, without limitation, the promoters from genes encoding seed storage proteins, such as napin, cruciferin, phaseolin, and the like (see U.S. Patent No. 5,420,034 to Kridl et al., which is hereby incorporated by reference in its entirety). Other suitable promoters include those from genes encoding embryonic storage proteins.

Promoters useful for expression in leaf tissue include the Rubisco small subunit promoter.

Promoters useful for expression in tubers, particularly potato tubers, include the patatin promoter.

In another embodiment of the present invention, expression of one or both transgenes is environmentally-regulated, i.e., through the use of an inducible promoter. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light. In some plants, it may also be desirable to use promoters which are responsive to pathogen infiltration or stress. For example, it may be desirable to limit expression of the hypersensitive response elicitor protein or polypeptide in response to infection by a particular pathogen of the plant. One example of a pathogen-inducible promoter is the *gst1* promoter from potato, which is described in U.S. Patent Nos. 5,750,874 and 5,723,760 to Strittmayer et al., which are hereby incorporated by reference in their entirety.

Expression of the transgenes in isolated plant cells or tissue or whole plants also requires appropriate transcription termination and polyadenylation of mRNA. Any 3' regulatory region suitable for use in plant cells or tissue can be operably linked to the coding regions in the transgenes. A number of 3' regulatory regions are known to be operable in plants. Exemplary 3' regulatory regions include,

without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

The promoter and a 3' regulatory region can readily be ligated to DNA molecules using well known molecular cloning techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference in its entirety.

In some instances, it may be desirable for the hypersensitive response elicitor to be secreted by the cells in which it is expressed into intercellular regions of the plant. Thus, it may be desirable to ligate a DNA molecule encoding a secretion signal to the coding region of the transgene coding for the hypersensitive response elicitor protein or polypeptide. A number of suitable secretion signals are known in the art and others are continually being identified. The secretion signal can be an RNA leader which directs secretion of the subsequently transcribed protein or polypeptide, or the secretion signal can be an amino terminal peptide sequence that is recognized by a host plant secretory pathway. Typically, the DNA molecule encoding the secretion signal can be ligated between the promoter and the coding region using known molecular cloning techniques as indicated above.

An exemplary secretion signal is the secretion signal polypeptide for *PR1-b* gene of *Nicotiana tabacum*. The DNA molecule encoding this secretion signal has a nucleotide sequence corresponding to SEQ. ID. No. 15 as follows:

```
cacgaagctt accatgggat tttttctctt ttcacaaatg ccctcatttt ttcttgtgtc   60
gacacttctc ttattcctaa taatatctca ctcttctcat gcccaaaact cccgcggrga  120
```

The polypeptide encoded by this nucleic acid molecule has an amino acid sequence corresponding to SEQ. ID. No. 16 as follows:

```
Met Gly Phe Phe Leu Phe Ser Gln Met Pro Ser Phe Phe Leu Val Ser
 1               5                  10                  15

Thr Leu Leu Leu Phe Leu Ile Ile Ser His Ser Ser His Ala Gln Asn
            20                  25                  30

Ser Arg Gly
        35
```

Once transgenes of the type described above have been prepared, they can be introduced into plant cells or tissues for subsequent regeneration of whole plants. Thus, another aspect of the present invention relates to a transgenic plant which has been treated or genetically modified so that the transgenic plant can either exhibit enhanced growth, disease resistance, stress resistance, or insect resistance to realize the maximum benefit of a value-added trait or otherwise overcome a yield penalty concomitant with a value-added trait.

According to a one embodiment, the transgenic plant of the present invention includes a DNA molecule encoding a transcript or a protein or polypeptide that confers a trait, wherein the transgenic plant or a plant seed from which the transgenic plant is grown, is treated with a hypersensitive response elicitor protein or polypeptide under conditions effective to impart enhanced growth, disease resistance, stress resistance, or insect resistance to the transgenic plant.

According to another embodiment, the transgenic plant of the present invention including a first DNA molecule encoding a transcript or a protein or polypeptide that confers a trait and a second DNA molecule encoding a hypersensitive response elicitor protein or polypeptide different than the protein or polypeptide encoded by the first DNA molecule. Because the transgenic plant includes at least two DNA molecules, the first and second DNA molecules can be inserted into a plant cell or tissue either individually (i.e., in separate constructs used during separate transformation steps) or simultaneously (i.e., in a single construct or in separate constructs used during a single transformation step).

Another aspect of the present invention relates to a system for use in transforming plants with multiple DNA molecules, typically although not exclusively during separate transformation events. This system includes a first DNA construct that includes a DNA molecule encoding a transcript or a protein or polypeptide which confers a trait to a host plant, and a second DNA construct that contains a DNA

molecule encoding a hypersensitive response elicitor protein or polypeptide which is different from the protein or polypeptide encoded by the DNA molecule of the first DNA construct. The first and second DNA molecules can be of the type described above. The first and second DNA constructs each contain a promoter operably linked 5' to the DNA molecule (e.g., first or second DNA molecule) and a 3' regulatory region operably linked to the DNA molecule.

A further aspect of the present invention relates to a DNA construct for use in transforming plants with multiple DNA molecules, typically during a single transformation event. The DNA construct includes a first DNA molecule encoding a transcript or a protein or polypeptide which confers a value-added trait to a host plant and a second DNA molecule encoding a hypersensitive response elicitor protein or polypeptide which is different from any protein or polypeptide encoded by the first DNA molecule. The first and second DNA molecules can be of the type described above. The DNA construct can include a first promoter operable in plant cells operably linked 5' to one or both of the first and second DNA molecules. Alternatively, where the first promoter is only operably linked to the first DNA molecule, the DNA construct can also include a second promoter operably coupled to the second DNA molecule. The first and second promoters can be the same or different. Generally, both the first and second DNA molecules will be ligated to a 3' regulatory region, which can be the same or different for each of the first and second DNA molecules.

Both the transgene or DNA molecule conferring a value-added trait and the transgene or DNA molecule encoding the hypersensitive response elicitor protein or polypeptide can be incorporated into cells using conventional recombinant DNA technology. Generally, this involves inserting the transgenes or DNA molecules into expression vector(s) or system(s) to which they are heterologous (i.e., not normally present). Because either single or multiple expression systems can be used, a single expression system can include a vector into which is inserted both the first DNA construct containing the first DNA molecule and the second DNA construct containing the second DNA molecule. Alternatively, the expression system can include two vectors into which are inserted one or the other of the first DNA construct containing the first DNA molecule and the second DNA construct containing the

second DNA molecule. The first and second DNA molecules can be ligated to the appropriate promoter(s) and 3' regulatory regions either before insertion into the expression vector(s) or system(s) or at the time of their insertion therein.

U.S. Patent No. 4,237,224 issued to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms, typically bacteria, and eukaryotic cells grown in tissue culture, typically plant cells.

As indicated above, several aspects of the present invention are directed to the preparation of transgenic plants. Basically, this is carried out by providing a plant cell (which may or may not already possesses a transgene), transforming the plant cell with one or more transgenes of the type described above under conditions effective to yield expression of such transgenes, and then regenerating the transformed cells into whole transgenic plants. Preferably the transgene(s) is stably inserted into the genome of the transformed plant cell and whole plants regenerated therefrom.

One approach to transforming plant cells with the transgenes or DNA molecules identified herein is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., which are hereby incorporated by reference in their entirety. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector(s) containing the DNA to be used in transforming the plant cell. Alternatively, the target cell can be surrounded by the vector(s) so that the vector(s) is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the

vector and DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the transgenes or DNA molecules identified herein is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the first and second transgenes or DNA molecules. Fraley et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference in its entirety.

The transgenes or DNA molecules identified herein may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference in its entirety. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the transgenes or DNA molecules. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the transgenes or DNA molecules identified herein into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with one or both of the transgenes or DNA molecules identified herein. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

The transgenes or DNA molecules identified herein can be introduced into appropriate plant cells by means of the Ti plasmid of *A. tumefaciens* or the Ri

plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells upon infection by *Agrobacterium* and is stably integrated into the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference in its entirety.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the transgene or DNA molecules identified herein. Suitable selection markers include, without limitation, markers coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection media containing an antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a transgenic plant of the present invention. Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference in their entirety.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major crop and medicinal plant species, trees, perennial and annual ornamental plants, and turf and ornamental grasses. Exemplary crop species include, without limitation, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, canola, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear,

melon, strawberry, cranberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Exemplary trees include, without limitation, maple, birch, oak, walnut, cherry, pine, and poplar. Exemplary ornamental plants include, without limitation, begonias, impatiens, geraniums, lilies, daylilies, irises, tulips, and roses.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the transgenes or DNA molecules identified herein are stably incorporated in transgenic plants, they can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the field. Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Alternatively, transgenic seeds or propagules (e.g., scion or rootstock cultivars) are recovered from the transgenic plants.

A further aspect of the present invention relates to a method of making a transgenic plant which includes providing a transgenic plant seed containing both the transgene or DNA molecule conferring the trait and the transgene or DNA molecule encoding the hypersensitive response elicitor protein or polypeptide, and then planting the transgenic seed under conditions effective to grow a transgenic plant from the transgenic seed. Although any medium can be used to germinate and grow the transgenic seeds, preferably they are planted in the soil and cultivated using conventional procedures to produce the transgenic plants. Preferably, the transgenic

plant seed is harvested from a transgenic parent plant as described above. Thus, the transgenic plants are propagated from the planted transgenic seeds under conditions effective to confer the value-added trait and hypersensitive response elicitor protein or polypeptide expression to subsequent generations.

Another method for preparing a transgenic plant of the present invention involves providing two distinct transgenic plant lines, one containing the transgene or DNA molecule conferring the trait stably inserted into its genome and the other containing the transgene or DNA molecule encoding the hypersensitive response elicitor protein or polypeptide stably inserted into its genome. The two lines are then crossed using conventional breeding techniques and the resulting generation segregated and self-crossed to propagate a single hybrid line which possesses the value-added trait conferred by expression of the first transgene or DNA molecule and expresses the hypersensitive response elicitor protein or polypeptide encoded by the second transgene or DNA molecule. Additional value-added traits can be crossed into such a transgenic hybrid line.

EXAMPLES

The following examples are provided to illustrate embodiments of the present invention, but they are by no means intended to limit its scope.

Example 1 - Increased Yields From Transgenic Cotton Varieties Treated With Messenger

Field trials designed to test the effects of Messenger® on disease resistance and crop yield in cotton were performed using the following transgenic cotton varieties: Delta and Pine Land ("DPL") 20B, DPL 33B, DPL 35B, DPL 50B, Stoneville BXN 47, and Paymaster 1220BR. All of the DPL cotton varieties are transgenic for genes encoding Bt toxin, which confers resistance to a specific class of insects. Stoneville BXN 47 is a transgenic cotton variety with a gene for resistance to the herbicide bromoxynil. Paymaster 1220BR is a transgenic variety with stacked transgenic traits: in addition to the Bt toxin gene, this variety carries a second gene conferring resistance to the herbicide glyphosate. The transgenic traits in all six

varieties have specific functions limited to providing insect resistance and/or resistance to herbicide. The transgenes are not intended to alter capacity for growth and yield so the characteristics of the non-transgenic parental varieties are retained in the transgenic varieties.

In the eight field trials where the cotton varieties were transgenic, Messenger® was applied by foliar spray or combined seed treatment and foliar spray. In five of these trials, different numbers of treatments and rates of application were tested. Yields were measured in lbs lint/acre or in lbs seed cotton/acre. The results of the trials are summarized in Table 2 below.

Table 2: Increased Yields From Messenger® Treated Transgenic Cotton

Trial	Cotton Variety	Treatment	Rate	Percent Yield Increase
1	DPL 20B	4 foliar	2.2 oz./acre	11.4
2	DPL 33B	3 foliar	2.2 oz./acre	11.4
3	DPL 33B	3 foliar	2.2 oz./acre	5.9
		3 foliar	4.4 oz./acre	9.5
4	DPL 50B	3 foliar	2.29 oz./acre	1.0
		3 foliar	4.59 oz./acre	15.0
5	DPL 35B	3 foliar	2.2 oz./acre	6.0
		seed and 3 foliar	2.2 oz./50 lb. 2.2 oz./acre	26.0
		seed and 3 foliar	2.2 oz./50 lb. 4.4 oz./acre	33.0
6	Paymaster 1220BR	3 foliar	2.2 oz./acre	4.7
7	Paymaster 1220BR	3 foliar	2.2 oz./acre	13.1
		4 foliar	2.2 oz./acre	11.8
		seed and 3 foliar	2 oz./cwt 2.2 oz./acre	11.5
		seed and 3 foliar	2 oz./cwt 4.4 oz./acre	16.2
8	Stoneville BXN 47	3 foliar	2.2 oz./acre	49.6
		4 foliar	2.2 oz./acre	60.4
		seed and 3 foliar	2 oz./cwt 2.2 oz./acre	14.0
		seed and 3 foliar	2 oz./cwt 4.4 oz./acre	38.8

DPL varieties 20B (trial 1) and 33B (trial 2), and Paymaster 1220BR (trial 6) were treated with spray applications of Messenger® at 2.2 oz./acre starting at the first true leaf stage, followed by early bloom and mid bloom applications. Yields from treated plants were higher than for the untreated control plants of the same varieties with increases ranging from 4.7% to 11.4%. Messenger® was applied at rates of 2.2 and 4.4 oz./acre for a second trial on DPL 33B (trial 3) and at rates of 2.29 and 4.59 oz/acre on DPL 50B (trial 4). Spray applications for these two trials were at first true leaf, early bloom, and mid bloom. In both trials, the high rate applications gave higher yields than the lower rate applications. The dose/response effect of Messenger® on yield is strong evidence that the observed yield increases were a direct result of the Messenger® applications.

In trials on DPL 35B (trial 5), Stoneville BXN 47 (trial 8), and a second trial with Paymaster 1220BR (trial 7), multiple types of treatments were carried out including foliar spray and foliar spray combined with seed treatment, with varying numbers of applications and application rates. Foliar applications were made at rates of 2.2 oz./acre or 4.4 oz./acre and from three to four times during the season. Multiple foliar applications were also made in combination with seed treatment at 2 oz./cwt or 2.2 oz./50lbs. Foliar Messenger® applications were made beginning at first true leaf, followed by early bloom and mid bloom applications. All treatments gave increased yields over untreated control plants of the same varieties. In each of these three trials, low and high rate applications were made and effects on increased yield showed a dose response correspondence with the amount of Messenger® applied.

The average increase in yield for all applications in all eight trials was 19.6%. For those trials with low and high rate applications, the average yield increases were 14.6% for low rate applications and 25.8% for the high rate applications.

Example 2 - Increased Fruit Number in Transgenic Cotton Varieties Treated With Messenger®

Cotton yields can be directly impacted by the total number of bolls produced per plant. In three of the eight trials presented in Example 1 (trials 2, 6, and 7), analysis of the effects of Messenger® treatment on yield were extended to include

a comparison of the numbers of bolls produced by treated and untreated transgenic cotton. The results are summarized in Table 3 below.

Table 3: Increased Fruit Number in Messenger® Treated Transgenic Cotton

				Plant Mapping 1		Plant Mapping 2	
Trial	Cotton Variety	Treatment	Rate	Fruit per Plant	Percent Increase	Fruit per Plant	Percent Increase
2	DPL 33B	control	-	8.73	-	10.2	-
		3 foliar	2.2 oz./acre	10.78	23.5	11.0	7.8
6	Paymaster 1220BR	control	-	38.1	-	14.8	-
		3 foliar	2.2 oz./acre	42.1	10.4	17.6	18.9
7	Paymaster 1220BR	control	-	10.5	-		
		3 foliar	2.2 oz./acre	11.1	5.7	-	-
		4 foliar	2.2 oz./acre	12.6	20.0	-	-
		seed and 3 foliar	2 oz./cwt 2.2 oz./acre	11.6	10.5	-	-
		seed and 3 foliar	2 oz./cwt 4.4 oz./acre	11.3	7.9	-	-

In trials performed with DPL 33B (trial 2) and Paymaster 1220BR (trial 6), plants received three spray treatments with Messenger®. Applications were made as described in Example 1. Two boll counts were made in each trial, once after early bloom and a second time near harvest. A higher number of bolls was present on treated plants in both trial 2 and 6, at each of the early and late season plant mappings ranging from 7.8% to 23.5 % increase in number over control plants.

A second trial performed with Paymaster 1220BR (trial 7) was carried out with four types of treatments, as indicated in Table 3. A late season plant mapping revealed increased boll numbers for all Messenger® treated plants compared with untreated Paymaster 1220BR plants, with increases ranging from 5.7% to 20.0%.

The six Messenger® treatments in the two trials resulted in higher yields than obtained from untreated control plants. The results of these trials indicate that an increase in boll number can be a contributing factor to increased yields obtained from Messenger® treated cotton. There is an important to distinction to be made between effects on yield from Messenger® and effects on yield resulting from transgenic traits conferred by insect or herbicide resistance genes such as those in the

transgenic cotton varieties in these trials. Such resistance genes do not increase the basic yield characteristics of the transgenic plant but simply reduce yield losses caused by insect or weed pressure. A combination of Messenger® and such resistance genes would have complementary effects on yield since Messenger® would provide a higher baseline yield through its effects on growth such as increased fruit number, while resistance genes such as Bt toxin would act to preserve that higher yield by reducing losses to insect pressure.

Example 3 - Increased Number of Open Bolls on Transgenic Cotton Treated With Messenger

The number of open bolls present at harvest is a factor in total yield. A trial including four different types of Messenger® treatments on the transgenic cotton variety Stoneville BXN 47 gave higher yields than obtained from untreated Stoneville BXN 47 (trial 8, Table 2). In addition to the measurements of overall yields, observations were extended to include a comparison of the numbers of open bolls at harvest on the Messenger® treated plants and untreated control plants. Four types of Messenger® treatments were tested in this trial. Two treatments consisted of either three or four foliar applications at rates of 2.2 oz./acre. The remaining two treatments consisted of seed application combined with foliar sprays using 2 oz/cwt for seed treatments and 2.2 or 4.4 oz./acre for three foliar applications. Open bolls were counted at three positions on the plants. Position 1 corresponded to the lowest node with bolls. Position 2 corresponded to the next node above on the stem. Position 3 included bolls at the third node and above combined into a single total. The totals for numbers of bolls at all three positions were also calculated. The results of this analysis are summarized in Table 4 below.

Table 4: Increased Number of Open Bolls From Messenger® Treated Transgenic Cotton

Trial	Cotton Variety	Treatment	Rate	Open Bolls Position 1	Position 2	Position 3	Per Plant	Percent Increase
8	Stoneville BXN 47	control	-	3.56	0.33	0.00	3.89	-
		3 foliar	2.2 oz./acre	5.11	1.78	0.00	6.89	77.1
		4 foliar	2.2 oz./acre	5.45	1.45	0.11	7.00	79.9
		seed and 3 foliar	2 oz./cwt 2.2 oz./acre	5.44	1.22	0.00	6.67	71.5
		seed and 3 foliar	2 oz./cwt 4.4 oz./acre	5.22	2.00	0.11	7.33	88.4

The four types of Messenger® treatments performed in the trial resulted in increased numbers of open bolls on Stoneville BXN 47 relative to untreated Stoneville BXN 47. Increases in open bolls ranged from 71.5% to 88.4%. A dose response effect of Messenger® treatment on open boll number was evidenced by a higher percentage increase in open bolls with applications made at a rate of 4.4 oz./acre compared to applications made at 2.2 oz./acre.

Example 4 - Increased Yield from Transgenic Cotton Grown in a Field Infested with Reniform Nematodes.

Nematodes are parasitic worms that live in the soil and attack the roots of cotton. In an infested field, reniform nematodes can cause a 10-25% loss in yield and as much as 50% loss under stress conditions such as drought. A field trial to test effects of Messenger® treatment on cotton under nematode pressure was conducted in a field known to be infested with reniform nematodes. The cotton variety in the trial was Stoneville BXN 47, identified in Example 1. Since the bromoxynil transgene cannot provide resistance to nematodes, this cotton variety is just as susceptible to damage by nematodes as non-transgenic varieties.

Messenger® treatments of four types were applied in this trial. Two treatments consisted of either three or four foliar applications at rates of 2.2 oz./acre. The two other treatments consisted of seed application using 2 oz./cwt combined with three foliar sprays at rates of either 2.2 or 4.4 oz./acre. Foliar applications were made at first true leaf followed by early and mid bloom applications. Yields from treated

and untreated plots of Stoneville BXN 47 were determined as well as nematode populations in the soil from the plots. Results are summarized in Table 5 below.

Table 5: Increased Yield From Messenger® Treated Transgenic Cotton Grown in Nematode Infested Field

Trial	Cotton Variety	Treatment	Rate	Nematode Population At Planting	Nematode Population At Harvest	Percent Change	Yield Increase
8	Stoneville BXN 47	control	-	9927	7609	- 23.4	-
		3 foliar	2.2 oz./acre	8889	6953	- 21.8	49.6
		4 foliar	2.2 oz./acre	8807	5948	- 32.5	60.4
		seed and	2 oz./cwt				
		3 foliar	2.2 oz./acre	6528	4867	- 25.4	14.0
		seed and	2 oz./cwt				
		3 foliar	4.4 oz./acre	10622	7957	- 25.1	38.8

Yields were substantially higher in all four plots receiving Messenger® treatment compared to untreated plots. The increased yields in response to Messenger® could be due to enhanced growth effects, induced resistance to nematodes, or a combination of both. Nematode populations declined over the course of the growing season with no significant difference in the amount of decline between treated and untreated plots, indicating that Messenger® did not directly affect nematodes. The significantly lower yield from the untreated Stoneville BXN 47 plot demonstrates the reserve potential for higher yield in a transgenic variety that can be elicited by Messenger®.

Example 5 - Application of Messenger® to Bt-transformed Corn Changes Toxicity Profile to Fall Armyworm

Non-Bt-transformed corn, (Yellow-sugary, 83-d maturity, cv. "Rogers", F1 Bonus, from Novartis) and Bt-transformed corn, (cv. "Rogers", GH-0937, also from Novartis) were planted in pots (one plant per pot, four replicate pots) and then placed in a greenhouse under normal conditions. When plants were 2-3 feet tall (pre-tassel), they were treated with a single foliar spray of Messenger® at a rate of 3 oz/acre in approximately 40 gal/acre. The concentration of $harpin_{Ea}$ (active ingredient) in this spray was approximately 17ppm.

Five days after the application of Messenger®, leaf discs of approximately 0.5 inch in diameter were collected from treated and non-treated plants and placed in on agar media in petri dishes. Fall armyworm (FAW, *Spotoptera frugiperda*) neonate larvae were added to each petri dish. Leaf discs were replaced as needed in order to provide a constant food supply to the larvae.

At 6 days after treatment (DAT), feeding activity by FAW was measured by counting the number of leaf disks completely eaten in both transformed and non-transformed corn, treated with and without Messenger®. As demonstrated in Table 6 below, substantial feeding activity occurred in both Messenger® and non-Messenger® treated, non-transformed corn. However, in Bt-transformed corn, very little feeding activity occurred.

Table 6 : Feeding Activity and Mortality Data for Fall Armyworm Feeding on Messenger® Treated and Non-treated Bt-corn and non-Bt-corn

		Feeding*	Mortality	
Corn Description	Treatment	6 DAT	7 DAT	8 DAT
Non-transformed	-	27	0%	0%
Non-transformed	Messenger®	34	0%	0%
Bt-transformed	-	2	30%	30%
Bt-transformed	Messenger®	0	80%	80%

* Number of leaf disks completely eaten by 20 larvae, with "0" indicating no leaf disks entirely eaten.
DAT = days after treatment.

At 7 and 8 DAT no larval mortality was recorded in non-Bt-transformed corn, whether treated with Messenger® or not. However, in Bt-transformed corn, mortality at both 7 and 8 DAT was substantially lower for Messenger®-treated compared to non-Messenger®-treated (Table 6).

The increased mortality of FAW in Messenger®-treated, Bt-transformed corn suggests that application of Messenger® may have synergistic effects at controlling larval feeding activity.

Example 6 - Herbicide Resistant Transgenic Crops

A variety of technologies have been developed for production of transgenic plants resistant to herbicides including glyphosate, Synchrony, glufosinate, sethoxydim, imidazolinone, bromoxynil, and sulfonylurea. Each of these

technologies relies on the introduction of a single gene that confers resistance to a particular herbicide. Since the introduced gene is limited to a single function, other agronomically important traits of the crop plants remain unmodified. Glyphosate resistant transgenic cotton, soybean, and canola, for example, are susceptible to the same range of diseases that affect the non-transgenic parental lines from which the transgenic lines were developed. Yield losses due to disease could be minimized by combining genes for herbicide resistance and hypersensitive response elicitor expression in the same transgenic plant, thereby allowing the full benefits of the herbicide resistance trait to be realized.

Example 7 - Insect Resistant Transgenic Crops

Bt toxin protein from the soil bacterium *Bacillus thuringiensis* has been used on crops for many years as a topically applied insecticide with activity against specific classes of insects. A large number of genes have been isolated that encode different versions of the Bt toxin protein with varying specificities in insecticidal activity. The introduction of a gene encoding Bt toxin into potato was one of the first commercial applications of transgenic technology in crop plants. Since then, the commercialization of insect resistant crops expressing Bt toxin genes has been extended to include cotton and corn, with other crops under development. The specific insect resistance function of the Bt toxin gene is generally effective, but disease resistance and growth traits remain unaltered in the transgenic crops expressing Bt toxin genes. While yield losses due to insect pressure are reduced in Bt toxin expressing crops, they are still vulnerable to losses caused by pathogens. Bringing Bt toxin genes together with a transgene coding for hypersensitive response elicitor expression would produce crops that are resistant to pathogens as well as insects. An additional benefit would be increased yield due to the enhanced growth effect of the hypersensitive response elicitor.

Example 8 - Transgenic Crops With Enhanced Nutrient Value

Transgenic technology can be used to modify the balance of nutrients in crops to eliminate nutritional deficiencies. Some food crops are naturally deficient in particular amino acids that are a necessary component of the human diet. Cereal crops are often poor in tryptophan and lysine while vegetable crops and legume crops such as soybean are low in cysteine and methionine. Amino acids present at low amounts can be increased to nutritionally useful levels through the introduction of a gene encoding a protein with a high content of a particular amino acid that is normally lacking. Another approach that allows improvement of nutritional value is modification of an existing biochemical pathway or introduction of a novel biochemical pathway by introduction of a transgene. This can result in production of a compound with nutritional value that is normally absent or present in low amounts. Rice is an important food crop worldwide but is naturally low in vitamin A. Transgenic rice with increased vitamin A content could help to alleviate dietary deficiencies in this nutrient and is currently being developed. Transgenes can also be used to modify fatty acid biosynthesis pathways so as to produce food oils with altered levels of saturation. This method of improving nutritional value has been applied to canola, soybean, and flax so far. An aspect common to all the above approaches for enhanced nutritional quality is that improvements to the crops are limited to nutritional characteristics. Disease resistance and overall growth and yield properties of the crops remain unimproved. Combining a transgene coding for hypersensitive response elicitor expression with genes that confer enhanced nutritional value would allow the generation of transgenic crops that maximize the nutritional advantages through reduced losses to diseases and through improved yields due to enhanced growth.

Example 9 - Compensation For Transgenic Trait-Associated Losses In Yield

Introduction of a transgene for a beneficial trait can on occasion result in the introduction of a disadvantageous quality. For example, evidence indicates that the glyphosate resistance trait by itself can result in reduced yield in crops expressing

the resistance gene. A study done at the University of Wisconsin compared 1998 yields from glyphosate resistant soybean crops with yields from non-transgenic varieties at multiple sites in 8 Midwestern states and New York. At a majority of sites, yields of the glyphosate resistant soybeans were significantly lower than the non-transgenic varieties. The growth enhancement effect of a hypersensitive response elicitor could act to decrease or eliminate the yield penalty if combined with herbicide resistance genes in transgenic plants.

Introduction of a transgene may on occasion result in the loss of an advantageous trait. New Mexico State University reported losses to fungal infection in transgenic cotton varieties during the 1998 cotton season. Paymaster varieties that were insect resistant due to the presence of a Bt toxin transgene were susceptible to *Verticillium* wilt. Since the non-transgenic varieties had been resistant to *Verticillium* wilt, the introduction of the Bt toxin gene had resulted in loss of the fungal resistance trait. Negative side effects on disease resistance that might result from introduction of a transgene could be reduced or eliminated by combination with a transgene coding for hypersensitive response elicitor expression, which actively confers a broad range of disease resistance.

Example 10 - Pathogen Resistant Transgenic Crops

Crops are subject to attack by viral, bacterial, and fungal pathogens. An extensive amount research has been devoted to identifying ways to make crops resistant to pathogen attack. As a result, a growing number of genes have been identified that confer or have potential to confer pathogen resistance when expressed in transgenic plants. A major limitation of the resistance genes characterized so far is they have restricted ranges of effectiveness. A gene may confer resistance to viral but not fungal or bacterial pathogens, and *vice versa*. In many cases the protection is more narrowly limited to a small subset of viral, bacterial, or fungal pathogens. Transgenic plants expressing any of these resistance genes have reduced susceptibility to attack by specific pathogens or classes of pathogens, but the narrow range of resistance leaves the plants vulnerable to attack by many other pathogens. An example of how a narrow range of protection conferred by a transgene can leave a

crop vulnerable to non-target organisms was demonstrated by substantial losses in Bt toxin cotton in Texas in 1996 to non-target pests. Hypersensitive response elicitor expression is effective in providing resistance against many viral, bacterial, and fungal pathogens. Combining the transgene coding for a hypersensitive response elicitor with resistance genes that are narrowly focused in transgenic plants would provide a broader range of protection and decreased losses.

Although the invention has been described in detail for the purposes of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.